Project Title: Spawning movements and habitat use of winter flounder in the southern GOM.

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Abstract:
With the exception of fish in the Georges Bank stock, it is widely believed that adult winter flounder (*Pseudopleuronectes americanus*) move inshore into estuaries and coastal embayments to spawn. During February 1 through May 31, dredging is prohibited shallow waters ≤ 5 m to protect the sticky, demersal eggs. However, there have been many indications that this paradigm may not apply in the Gulf of Maine (GOM). To understand winter flounder spawning movements and habitat use in the southern GOM more clearly, 40 pre-spawning adult fish were acoustically tagged offshore and tracked in 2009. In addition, a bottom trawl survey was conducted in the offshore study area to quantify how the reproductive status of the general population changed over time.

Peak spawning of winter flounder in Ipswich Bay occurred in late April to early May. Only six fish (16%) were detected entering estuaries between the end of April and August indicating that the majority of the tagged fish did not spawn in estuaries but remained in deeper, coastal waters. Surveys made within a NH estuary when one tagged female was present (May-June) revealed that all adults present had already spawned and were actively feeding.

As of September 2011, conventional tagging returns (395 fish tagged, 5% return rate) show both long and short movements. Fish have been recaptured approximately 0.6 to 57.2 km from their tagging sites in depths of 2 to 75 m. Days at liberty range from 33 to 453 days, with an average of 171 days. Spawning site fidelity is evident from inter-annual recaptures of tagged winter flounder. However, relocations of fish well outside of Ipswich Bay suggest mixing of populations of winter flounder is likely. Based on the results of this study, consideration should
be given to extending EFH for GOM winter flounder eggs to deeper waters to protect the predominant coastal spawning populations.

Introduction:

Winter flounder, *Pseudopleuronectes americanus*, is a commercially and recreationally important demersal flatfish found along the northwestern Atlantic coast; ranging from Georgia, USA to Labrador, Canada (Scott and Scott, 1988); it is most common from Nova Scotia to New Jersey (Perlmutter, 1947). It is a long-lived flatfish and can reach a maximum age of 15 years and a maximum length of 58 cm (Fields, 1988). Winter flounder are federally managed as three stocks: the Gulf of Maine (GOM) stock, Georges Bank (GB) stock, and Southern New England/Mid-Atlantic (SNE/MA) stock (Pereira, 1999). As with most groundfish species, catches have declined precipitously in recent years. Total commercial landings of all three stocks in 1981 were about 17,575 mt. Since then, catches have declined dramatically to 1,858 mt in 2010 ((NEFSC), 2011).

The general decline in fisheries resources, including those for winter flounder, prompted regulators to broaden their view of management beyond simply regulating fishing effort. In particular, there is a recognition that “the long term viability of living marine resources depends on protection of their habitat” (NMFS Strategic Plan for Fishery Research). This resulted in formalization of the concept of Essential Fish Habitat, defined by Congress as “those waters and substrate necessary to fish for spawning, breeding, feeding or growth to maturity”. Consequently, the Sustainable Fisheries Act, signed in 1996, required the eight regional fishery management councils to: 1) describe and identify essential fish habitat (EFH) in their regions; 2) take actions that conserve and enhance the EFH; and 3) minimize the adverse effect of fishing on EFH as they develop their fishery management plans. Given this federal requirement, the National Marine Fisheries Service has prepared a series of Essential Fish Habitat Resource Documents, including one for winter flounder authored by Pereira et al. (1999) and updated in 2004 (Pereira, 2004).

In the winter flounder EFH resource document, Pereira et al. (1999) summarize the movements of adult fish during the winter-spring spawning season with the following statement: “With the exception of the Georges Bank population, adult winter flounder migrate inshore in the fall and early winter and spawn in late winter and early spring. Following spawning, adults typically leave inshore areas...” While this has been well documented for SNE/MA populations through tagging studies (Howe and Coates, 1975; Perlmutter, 1947; Phelan, 1992; Saila, 1961), this may not be the case for the GOM populations of winter flounder. Indeed, the GOM stock appears to have a fundamentally different spawning habitat from the more well-studied SNE/MA stock.

Few studies have examined adult winter flounder movements in the GOM, and most of these were conducted over 30 years ago (Howe and Coates, 1975; McCracken, 1963; Perlmutter, 1947). Both McCracken (1963) and Howe and Coates (1975) noted the direct contrast of winter flounder seasonal movements north and south of Cape Cod. South of Cape Cod, the SNE/MA stock migrates inshore in the late fall/early winter to spawn in the estuaries, and then moves back offshore in early spring, identical to what is reported in Pereira et al. (1999). North of Cape Cod, in the GOM, fish remained offshore
during the winter and underwent limited seasonal migrations. None of these earlier studies addressed spawning locations, which have not been documented for winter flounder north of Cape Cod. There are, however, several lines of evidence that winter flounder do not spawn in the estuaries along the Gulf of Maine. These include:

1) The trawl surveys, that have been conducted just outside of the Hampton-Seabrook Estuary (HSE) along the NH/MA coast by Normandeau Associates, Inc. (NAI) for >20 years, have caught few adult winter flounder, and even fewer in spawning condition. In 2006 for example, only five adults were caught in the spring, and four of these had already spawned ((NAI), 2006). The one ripening female was acoustically tagged and released by Walsh and Howell (2007), but never entered the estuary.

2) Few adults have been found in either the HSE (Fairchild et al., 2008) or Great Bay Estuary, NH (Armstrong, 1997). These findings suggest that the vast majority of adults and spawning fish are somewhere other than the estuaries associated with the southern GOM.

3) Commercial fishermen report, and UNH scientists have documented, that spawning winter flounder are found in offshore locations. From 1995-2011, pre-spawning gravid winter flounder have been caught each March in the southern part of Jeffrey’s Ledge for related research projects (Fairchild, unpubl. data).

4) NOAA’s Industry Based Survey (IBS) indicates that the largest aggregations of adult winter flounder caught during the pre-spawning months are not found near the mouths of estuaries, but rather in coastal locations further offshore (B. Hoffman, Mass. Div. of Mar. Fish., personal communication).

5) More importantly, DeCelles and Cadrin (2010) acoustically tracked 72 adult winter flounder during 2007 and 2009 in Plymouth Bay and Estuary, in MA north of Cape Cod, and found that the fish displayed two movement patterns during spawning season. A small group migrated into the estuary, but the majority of tagged winter flounder remained in the coastal bay waters.

In the updated winter flounder EFH document (Pereira 2004), “Further research into defining spawning areas...” is recommended. “The full range of depths and sediment types used by spawning winter flounder has not been adequately described.”

**Project Objectives:**

The overall goal of this study was to determine if winter flounder in the western GOM, particularly in Ipswich Bay, are spawning offshore rather than in estuaries by: 1) mapping the spatial distribution and movements of adult winter flounder during the spawning season using telemetry; 2) sampling the adult winter flounder from March to June to quantify how the reproductive status of these fish changes over time in an offshore study area; and 3)
determining how the spatial distribution of spawning fish relates to attributes of the spawning habitat.

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**Methods:**

**Study Area**
Winter flounder were studied in Ipswich Bay, in the southwestern Gulf of Maine (Fig. 1). The 35 km² offshore area, ranging from 40-60 m deep, was selected for several reasons. First, it was one of several places identified by fishermen as an area where adult pre-spawning winter flounder are abundant. Second, it has a variety of substrate types, varying from sandy mud to gravel to exposed ledge, most of which has been reported as suitable for winter flounder spawning (Bigelow and Schroeder, 1953; Crawford and Carey, 1985; Pereira et al., 1994; Pereira, 1999; Schultz et al., 2007). Third, it is in close proximity to several estuaries (Plum
Island Sound (PIS), Merrimack River, HSE, Great Bay Estuary) such that it was feasible for winter flounder to move into estuaries for spawning.

**Trawl Surveys**

Bottom trawling was conducted by commercial fishing vessels in Ipswich Bay from March through June, 2009 to tag adult winter flounder and assess abundance and spawning condition of the population over time. A total of 83 30-min tows were made over 15 days using 16.5 cm codend mesh nets. Trawling was conducted 5–19 km offshore and spanned a 206 km² area, bounded by the latitudes 42° 43.34’N and 42° 54.57’N, and longitudes 070° 35.04’W and 070° 45.64’W (Fig. 1). Bottom depth at trawl sites ranged from 29 to 91 m, with a mean depth of 59 m; bottom type was mud, sandy-mud, sand, and gravel. Supplementary trawling was conducted inside the Hampton-Seabrook Estuary (HSE) using the R/V Red Cloud and a small, 5 m otter trawl on May 19, June 2, and June 16. In total, nine 15-minute tows were completed in 1–5 m of water over sandy bottom.

All captured winter flounder were measured, marked with conventional external tags (yellow 2 cm disc tags for fish > 19 cm or 5 cm t-bar tags for fish < 19 cm), and their sex and reproductive condition (immature, developing, ripe, running ripe, or recovering) determined by external inspection or ovarian biopsy. Forty pre-spawning winter flounder were tagged with acoustic transmitters (see below). All bycatch was identified and enumerated, and all catch, expect where noted, was released alive at the surface after each tow.

**Ovarian biopsies**

To verify reproductive status of female winter flounder, a gonad biopsy was attempted with most fish considered to be potentially female and in developing or spawning condition. Similar to Asturiano et al. (2000) procedures, a scalpel was used to make an initial 0.5 cm incision in the body wall over the ovaries, and a 16 gauge needle attached to a syringe was inserted through the opening and used to extract eggs from the ovaries. All oocytes within a sample were counted and mean egg size (diameter) was calculated using a random subsample of at least 10% of the total eggs in that sample (no statistical differences in mean size were found between 10% and 100% of eggs from 9 different samples; ANOVA p>0.05). Oocyte diameter and characteristics were used to stage ovarian development according to Burton and Idler (1984). Biopsies were not attempted on any females that were tagged with acoustic transmitters in order to minimize physical trauma.

**Sex and maturity staging**

Females were positively identified if the fish was mature, and in its pre-spawning, spawning, or immediate post-spawning phase, or oocytes were present in gonadal biopsies. Males could be identified only if they extruded milt. Females were classified as developing (showing small distinctive bulge in the abdominal cavity posterior to the genital pore), ripe (large distinctive bulge in the abdominal cavity posterior to the genital pore), running ripe (extruding eggs), or spent (showing a conspicuous concave depression in the gonadal area), or recovering (resting). If a flounder displayed none of these signs, and either no eggs were obtained from or there was not a biopsy attempt, then its sex was considered undetermined.
This undetermined group included sexually immature fish, mature fish that had already spawned and physically recovered, and potentially mature fish that could have skipped spawning during that year. Differentiating between mature and immature fish of unknown sex was difficult because of individual variation in size at maturity and a general difference in size at maturity between males and females each year. For flounder of unknown sex < 28 cm TL, fish were classified immature. This size was based on the average of the two mean lengths at 50% maturity for males (27 cm) and females (29.7 cm) from past surveys (1982-2007) of the inshore western GOM stock (O’Brien et al., 1993; P. Nitschke, NEFSC, unpublished data).

Acoustic Tags and Receivers

To study movements and fine scale distribution of winter flounder within Ipswich Bay during the spawning season, 40 pre-spawning adult winter flounder (18 males, 22 females) were acoustically tagging with Vemco V9P1L transmitters. Each tag weighed 2.7 g in water, was 9 x 46 mm in size, had approximately a 12 month lifespan, and transmitted a distinctive series of pulses that identified individuals. These pulses had a 180 s delay to avoid “code collisions” and extend battery life for 1 yr. Because adult flounder > 1 kg were tagged, the weight of the tag was well below the maximum of 2% fish body weight recommended for aquatic organisms (Winter, 1983). These identifying coded pulses (locations) were detected by stationary, submersible, single channel receivers (Vemco VR2W) when the tagged fish came within range; each record was time stamped. Because the acoustic tags contained a pressure sensor, these receivers also recorded the depth history of each fish. Forty-four stationary receivers initially were positioned 475 m – 800 m apart from their nearest neighbors in a grid design (similar to Fabrizio et al.’s (2005) design) in the area where the fish were tagged and released (Fig. 2a). This initial array was approximately 20 km² in size and 8 km from shore on its western edge, and ranged from 40 to 70 m deep. Receiver detection ranged from 250 to 400 m in the open ocean depending on weather conditions. Each receiver was moored using a system consisting of a 12 kg Danforth anchor attached via a 5 m chain to a 45 kg weight. The stationary hydrophone was attached to a buoyed line in the middle of the chain so that it floated 5 m off the bottom. Another line led from the weight to a high flyer at the surface. In addition to the offshore receiver array, multiple overlapping hydrophone receivers were deployed in March 2009 at the mouth of each adjacent estuary as an “acoustic gate” so that any acoustically tagged fish that entered was detected. A total of 27 receivers were placed from Kittery, Maine to Gloucester, MA, and covered the entrances to Portsmouth Harbor, Little Harbor, Hampton-Seabrook Estuary, Merrimack River, Plum Island Sound (PIS), Ipswich River, Essex River, and the Annisquam River (Fig. 1). Depth ranged from 2 to 20 m at these inlets. Minimum receiver detection in these shallow estuaries was 100 m, therefore, receivers were spaced 200 m apart.

A hand-held directional hydrophone and portable receiver (Vemco VR100), capable of detecting the fish’s location (GPS), also were used. When the position of each acoustic tag (fish) was found using the handheld directional hydrophone, location, date, time, depth, substrate type, and notable bathymetric features were recorded.

Acoustic Tagging and Tracking
Winter flounder were mostly absent in and near the study area until early April when a significant amount of winter flounder were caught, indicating that their seasonal inshore migration was underway. Over 3 days (April 5-6, 11), a total of 18 males and 22 pre-spawning females were caught in the same general area, tagged with transmitters, and released at a common, central location to facilitate effective tracking (Table 1). The release site was 11 km from shore, 66 m in depth, and located at 42°52.077'N, 70°40.047'W, directly east of Seabrook, NH (Fig. 2).

Fish were captured in 30 min tows and placed in flowing seawater tanks onboard the vessel. Only visibly ripening females (using the criteria of O’Brien et al. 1993) and ripening males (milt in the cannula) were tagged. Acoustic tags were attached externally, mounted in a silicone tubing saddle, and then pinned to the epaxial muscles according to the protocol used by Fairchild et al. (2009) and DeCelles and Cadrin (2010)(Fig. 3). Previous laboratory studies with adult winter flounder showed that tag retention rate for larger (V13) tags was at least four months and that swimming behavior was not affected (Walsh, unpublished data). More importantly, the tags did not impede spawning ability as several similarly tagged females spawned in the laboratory during this study. All acoustically tagged fish were held in tanks on board the vessel to allow for observation and recovery, and only those that appeared to be robust were released.

During tag and release, the off-shore receiver array was deployed around the release site in the study area (Fig. 2a). In addition, manual tracking with a handheld hydrophone began April 10 and continued several times/week thereafter until the end of June. Searching occurred in a grid pattern (0.5 km intervals) centered on the last known position of each tagged fish, similar to the methods used for cod by Robichaud and Rose (2001) and Siceloff and Howell (this issue). Total estimated area covered by manual tracking was 234 km$^2$ based on a detection range of 650 m over 25 days (Fig. 4). All stationary receivers were downloaded periodically to validate the direction of fish movements. Because the tagged flounder displayed non-random movement, the array was moved inshore on April 29 to continue passive tracking of the majority of the fish (Fig. 2b). On May 30, all offshore receivers were retrieved. The 27 estuarine receivers continued to serve as acoustic gates until Sept. 15.

Study area designs, trawl locations and tag recaptures were mapped using Nobeltec VNS Max Pro chart software. Acoustic telemetry tracks were plotted and analyzed in ArcGIS 9.3 using Hawths Tools. Sigmaplot 2000 was used to plot catch data over time and depth profiles from acoustic tags. Bottom depth was obtained in ArcGIS from a raster map of Gulf of Maine bathymetry derived from USGS digital sounding data.

Results and Conclusions:

Offshore Surveys

A total of 397 winter flounder were caught in 92 tows in March-June 2009 in the offshore study area. Fish size ranged from 17 – 46.5 cm TL (mean = 31.3 cm, S.E. = 0.26; Fig. 5). Females typically were larger than males: females ranged from 25 – 46.5 cm (mean = 33.8 cm, S.E. = 0.29), while males were 22 – 37 cm (mean = 29.8 cm, S.E. = 0.58). Mature fish of undetermined sex were 28 – 41.5 cm (mean = 31.7 cm, S.E. = 0.38), while immature fish were
17 – 27.5 cm (mean = 24.1 cm, S.E. = 0.31). The mean numbers of ripe and developing fish caught per tow increased over time in March and April and peaked in late April and early May, while the number of spent females peaked slightly later, in early and late May (Fig. 6). By late May, the majority of flounder showed no signs of spawning and sex was not identifiable externally (Fig. 6). In addition, five females in running ripe condition were caught in the offshore area between April 6 and June 9.

Gonad biopsies were successfully taken from 87 females. Mean oocyte diameters varied from 0.60 – 0.95 mm/fish with low variation/date (Table 2), indicating all ovaries were post-vitellogenic. With the exception of larger eggs from fish caught on May 8 (Kruskal-Wallis, p<0.01), mean egg size was constant across time. To verify staging techniques, seven egg samples spanning a range of mean egg sizes (0.6 – 0.91 mm) and collection dates (March 3-4, April 24, May 8) were sectioned and stained. Histology verified that all seven females were in “ripe” reproductive condition; most oocytes were hydrated.

Most winter flounder caught at sea (94%) were tagged: 336 were tagged with disc or t-bar tags and 40 with acoustic transmitters. Twenty-one fish were not tagged: 17 were considered too small, and four were in poor condition.

**Estuarine Surveys**

In total, 172 flounder were caught in the HSE. Due to the smaller mesh size, as well as the location and habitat, the majority of fish caught (88%) were juveniles <20 cm long. Total length ranged from 6.5 to 46 cm TL (mean = 14.6 cm, S.E. = 0.60). A total of 21 winter flounder were disc tagged.

Most fish from the HSE surveys were considered immature (n=154), and, except for 4 spawned out females (range = 33.5-46 cm TL; mean = 37.6 cm TL), all large fish (n=14; range = 28.5-35 cm TL; mean = 32 cm TL) were of undetermined sex (i.e. showing no signs of spawning). Nine of these fish were sacrificed and verified through dissection as females. In addition, crude stomach dissections revealed that 89% of these females (n=8) had full stomachs and were feeding; prey consisted of cumaceans, sand lance (≤ 41 mm TL), whole clams (8-12 mm), clam siphons, polychaetes, and whole cancer crabs (10-12 mm CW).

**Tracking**

Two out of forty tracked flounder (Fish 690 & 720) did not move significantly from their release site throughout April. These were considered mortalities and excluded from all subsequent analysis, leaving 38 fish (17 males, 21 females). Of these, 61% (n=23) moved outside of the original array in less than three days after release in early April, and 84% (n=32) were outside of it in less than a week. In addition, 29% (five females, six males), not only left the array quickly, but were never found again after mid-April (Table 1: Fish 683, 689, 691, 698, 700, 702, 705, 707, 710, 714, & 715).

Initial headings out of the array were in all directions, though approximately half (n=16) initially moved towards shore (West/NW/SW) as they left the array. The initial direction of flounder movements after tagging and release was not a reliable predictor of each flounder's overall track, and 15 flounder were observed to reverse their initial course. Some were tracked doubling back and passing across the entire array in the opposite direction in late April or May,
and several appeared to move around the array entirely and were later relocated on the opposite side of the array from where they left.

**Inshore Movements**

Overall, 68% of fish (n=26) were tracked moving inshore during the study period (April - June), by a combination of westward movements across the original array, manual relocations inshore of their release site, detections on the two inshore receiver lines in May, or detections on estuary receivers (Fig. 7). At the beginning of May, 55% of fish (n=21) were found to be present in Ipswich Bay or its estuaries; by late May, 45% (n=17) were still present. All were tracked moving inshore of their release, and most fish tracked in May (n=18) moved into water <30 m in depth. Fish were found as shallow as 8 m deep along the beach (Fish 699), or shallower in estuaries (Table 1, Fig. 8). For fish that did not enter estuaries (n=32), the shallowest depth where fish were located ranged from 8 to 68 m (Table 1). Mean shallowest depth was 25 m (± 8 m) for fish tracked past mid-April (and excluding those that entered estuaries). Relying on estuarine receivers and manual tracking, nine fish were relocated in Ipswich Bay in June - five in estuaries and four (Fish 685, 699, 704, & 709) near shore around Hampton Shoal Ledge (HSL; Fig. 7). Despite search efforts up and down the coast, these four fish were the only fish relocated in Ipswich Bay in June.

**Estuarine Movements**

Six fish (16%) were detected entering estuaries between April and August 2009 (Fig. 8, Table 4). One fish entered the HSE, two entered the Merrimack, two entered PIS, and one entered Essex Bay. No fish were detected in the Piscataqua/Little Harbor area or the Annisquam River. The two fish found in the Merrimack were male, while the rest were female. Both Fish 695 and 696 left and entered their respective estuaries during the day, but the other four fish all entered the estuaries and left them at night. Half of the fish stayed in the estuaries for < 4 days while the other half remained in the estuaries on average for 102 days (Table 4). One fish (711) that entered PIS, continued to be heard by UMASS receivers until mid-November when their receivers were removed. Another fish (682) was never detected leaving the estuary (HSE) and it is unclear whether it actually left; it is possible that it moved into the farther corners of the estuary or was caught. There appeared to be no clear pattern between fish that occupied estuaries for short or long durations.

With the exception of Fish 682 & 719, fish tracks show that these fish moved directly from the offshore study area into estuaries (Fig. 8b-e). However, the offshore receiver array was not shifted inshore until April 29 (Fig. 2) so it is unlikely that these tracks are complete. Both Fish 682 & 719 travelled to HSL before moving into estuaries (Fig. 8a,f). Fish 682 was detected on HSL for 16 days from April 18 to May 4. Fish 719 was detected for 1 day on May 21 in 7m water at HSL.

**Offshore and Southern Movements**

Three flounder (8%), all females, were relocated south of the Ipswich Bay study area by receiver arrays deployed by Massachusetts Division of Marine Fisheries. They were detected
along the southern coast of Cape Ann in Massachusetts Bay, and the southern edge of Jeffreys Ledge.

Fish 701 moved northwest then backtracked southeast across the original array in mid-April and left the study area heading southeast. The night it moved out of the array, it rose at least 45 m above the seafloor (~27 m depth) before passing out of detection range. On June 12, almost 2 months later, it was found 22 km to the southeast on southern Jeffreys for 24 h. It briefly reappeared again on southern Jeffreys a month later on July 17, 2 km to the west.

Fish 712 moved immediately out of the original array heading northeast after the April 6 release. Almost 4 months later, on July 26, it was relocated 32 km away on the southern side of Cape Ann. It reappeared briefly 12 km northeast on southern Jeffreys on August 5, and again sporadically August 19-28.

Fish 718 initially was tracked moving inshore, less than 4 km from its release, and edged gradually south before disappearing in late April. It was relocated on July 26, three months later, for two days on southern Jeffreys.

Recaptured Fish

As of September 2011, 20 tagged flounder were recaptured and reported by fishermen (5% return rate from 395 tagged fish; Table 3). The majority (n=16) were recaptured in 2009 with the remainder (n=4) captured in 2010. Recaptured fish were at liberty for 33 to 453 days after tagging with an average of 171 days. Three flounders (601, 610, 689) tagged within the HSE were recaptured near their release locations inside the estuary after 36, 396, and 445 DAL (days at liberty). The rest of the recaptured fish (n=17) were tagged at sea. Ocean-released fish were recaptured from 0.6 to 57.2 km from their tagging sites, with a mean of 14.5 km (net displacement), and were recaptured in depths of 2 to 75 m. Most were recaptured in Ipswich Bay (n=10). Five fish (469, 524, 590, 599, 931) tagged in the offshore area were recaptured in or at the entrance of estuaries. Two fish were recaptured outside of Ipswich Bay in Massachusetts Bay – one on Stellwagen Bank (Fish 908), the other in Salem Sound (Fish 671).

Thirty-one percent (31%) of fish recaptured in 2009 were within 7 km from their release sites. All fish recaptured the following year were located close to original release locations, ranging from 0.6 to 16.7 km (mean = 7.2 km). Two of these (Fish 599 & 689) were mature females.

Spawning location – in estuaries or offshore?

Given that only 16% of acoustically tracked winter flounder entered estuaries during the spawning season and no ripe fish were caught in estuarine trawl surveys, it is apparent that in Ipswich Bay, winter flounder are not estuarine-dependent spawners. When tagged, all tracked female and male fish were ripe and running ripe, respectively. Laboratory studies proved that the acoustic transmitters did not impede spawning. Therefore, as evident from tracks of the remainder of acoustically tagged fish, the majority of the population spawns in deeper, coastal or offshore waters. Fish that did move inshore, were found only as shallow as 25 m (± 8 m) on average. The shallowest non-estuarine location was 8 m at HSL. Forty-two percent (42%) of the fish were never found shallower than 44 m; many of these fish never moved inshore either.
Because some (n=6) winter flounder were tracked entering estuaries during spawning season, it is possible that they did spawn in those estuaries. Spawning behavior in the wild has never been described, but several have described spawning behavior in captivity (Breder, 1922; Stoner et al., 1999; Fairchild unpublished data). All witnessed nocturnal spawning events. Breder (1922) noticed that fish, especially females, had an increase in swimming activity prior to spawning. In a large (121,000 liter) research aquarium over 60 days, Stoner et al. (1999) found that females spawned up in the water column an average of 40 times, and males 147 times. Males initiated spawning events, often resulting in spawning by as many as six secondary males; strictly paired spawnings were uncommon (22%). Fairchild observed that in shallow tanks (200 liter), females still exhibited increased swimming and off-bottom activity during spawning events. Unfortunately, in shallow estuaries, these events are difficult to discern via telemetry, even with tags containing pressure (depth) sensors. Winter flounder tracked in the estuaries did not show such patterns. However, due to 3 m tidal variation, limited range of estuarine receivers, and brevity of spawning activity (<2 min), it is unlikely that such a brief event using transmitters coded for 3 min delays would be recorded. In addition, there are limited detections for 4 out of 6 estuarine fish between the dates they moved west out of the ocean receiver array and into the estuaries as their inshore movements occurred before April 29 when the offshore array was reconfigured (Figs, 2, 8). Like Fish 682 & 719 that were detected at HSL before they moved into estuaries (Fig. 8), these other estuarine fish may have had similar pre-estuarine movements that were not detected.

It is apparent from the many movement trends seen in this study that winter flounder employ several different reproductive strategies, especially in the southwestern GOM. This confirms DeCellies and Cadrin’s (2010) observations of adult winter flounder in another southern GOM system, Plymouth Bay and Estuary, during 2007-08. Similar to this study, they used acoustic telemetry to monitor 72 fish and identified two groups of winter flounder based on location (bay, estuary) during the spawning season (March – May); the majority (76%) was located in the bay. Like the Ipswich Bay study, most (n=8) of the tagged gravid females did not enter Plymouth Estuary showing that winter flounder do not rely on estuaries for spawning. However, Plymouth Bay is a smaller and more contained area than Ipswich Bay. This Ipswich Bay study provides more details on depth ranges occupied by spawning winter flounder and clearly identifies that, contrary to established paradigms, some GOM winter flounder populations spawn in deep, offshore areas.

Spawning habitat of winter flounder

Little is known of the fine scale spawning habitat of winter flounder, and all studies except for this one and DeCellies and Cadrin’s (2010) have occurred exclusively in estuaries or harbors. Predicting spawning habitats have been based on the location and environmental tolerances of adult fish or eggs. South of Cape Cod, spawning appears to occur in areas of estuaries where larvae would be minimally displaced by tidal movement (Crawford and Carey, 1985). Scarlett and Allen (1992) and Stoner et al. (1999) found the distribution of spawning males and females overlapped only in the middle portions of two New Jersey estuaries; from these trawl surveys, they surmised that most spawning occurred in these areas. They speculated that the selective advantage of spawning in the area might have been associated
with favorable temperatures, salinities, hydrodynamic properties, or sediments. McCracken (1963) found that adults died at 8 ppt salinity, and suggested that upstream, spawning migration is probably limited. Water temperatures in these estuaries during the spawning season vary from 1 to 10 °C (peak spawning at 2 to 5 °C), and salinities range from 10 to 35 ppt (Bigelow and Schroeder, 1953; Peary, 1962; Scarlett and Allen, 1992). Despite using acoustic transmitters on gravid females, DeCelles and Cadrin (2010) were not able to pinpoint spawning locations of winter flounder in Plymouth Estuary and Bay either. By using acoustic gates, they could determine whether females were in the estuary or the bay during spawning season, but not exact locations.

Since winter flounder eggs (0.75-0.85 mm diameter) are demersal and adhesive (Bigelow and Schroeder 2002), their location can be used as a predictor for spawning location. Winter flounder do not appear to be substrate-specific spawners. Crawford (1990) reported that females deposit their eggs on gravel bars, algal mats, eelgrass beds, and near freshwater springs. Using a benthic sled, Pereira et al. (2006) located 164 winter flounder eggs in a CT harbor on fine mud habitats. According to the winter flounder EFH document, eggs typically are found in shallow water (< 5 m) with bottom water temperature of <10°C and salinities of 10-30 ppt (Pereira at al. 1999).

During this study, the only winter flounder that were located in water <5 m deep, were those that entered the estuaries; this depth range is not a good predictor of winter flounder spawning locations in the southwestern GOM. Areas where spawning males and females overlapped were identified in Ipswich Bay from both trawl surveys (29 to 91 m depth) and from tracked fish (8 to 66 m depth). In these areas, substrate included mud, sand, gravel, rocks, and ledge.

One of the limitations of this study was relying on trawling for sampling the winter flounder population. Tows could only be conducted in areas without obstructions to the net (e.g. mud, sand, gravel bottoms), and thus, certain areas (e.g. rocky bottom, ledges, narrow gullies) were either under- or not represented in the surveys. However, acoustic tracking compensates for some of these deficiencies.

During May through June, 21% of the fish (n=9) were found in and around HSL, including two fish (682 & 719) that were relocated there before entering estuaries (HSE and PIS). It is clear that HSL is an important winter flounder area, and may be a spawning location for these fish. HSL is an area of vertical relief located directly west of the tagging area and release site, and several fish appeared to pass across this area or take up temporary residence there in May and June in the gullies and pits between elevated features or along the ledge's northern and eastern slopes. It was difficult to obtain a clear signal and successfully identify tags when conducting manual tracking in such structurally complex areas, likely because flounder were often on the bottom and their transmitter was blocked by elevated bathymetry. Nevertheless, four fish were found there sporadically throughout May and June until the study ended.

Winter flounder movements

Relatively few studies have examined adult winter flounder movements in the GOM through tagging, and most of these were conducted over 30 years ago (Perlmutter 1947; McCracken 1963; Howe and Coates 1975). There is evidence that northern winter flounder
populations undergo shorter migrations than southern populations, which would favor discreet spawning populations. In a 10-year tagging study at 21 locations off Massachusetts, Howe and Coates (1975) found that north of Cape Cod, movements of winter flounder were typically localized and confined to inshore waters within 10 km of release locations. For example, recaptured adult winter flounder that were tagged and released in Boston Harbor during 1960 and 1961 traveled an average distance of 3.4 and 2.2 km, respectively. East and south of Cape Cod, recaptured winter flounder indicated a seasonal movement over average distances of 5.7 km to 61.2 km in a southeastward direction related to water temperature-depth contours. In addition, little mixing occurred between GB and inshore GOM fish. Furthermore, other studies have shown that winter flounder are divided into distinct localized populations associated with the specific bays and estuaries in the northeast Atlantic coast (Klein-MacPhee 1978), and that water temperature plays an important role in governing seasonal movements. Over 10 years, 637 adult winter flounder were tagged in the GOM in Boothbay Harbor and Johns Bay, Maine; the majority of recaptured fish were recovered in the area in which they were tagged, regardless of whether they were tagged in inshore or offshore areas (Perlmutter 1947). McCracken (1963) examined fish in multiple bays in New Brunswick and Nova Scotia, Canada. In some bays, adults were located inshore during the winter and spring seasons but as water temperatures warmed above 15 °C, they moved out into offshore waters in the summer and fall seasons. In contrast, adults in other bays remained inshore all year where water temperatures usually were < 14 °C. Further north, adult winter flounder remained in discrete areas in Conception Bay, Newfoundland during the peak foraging season except when they moved to deeper waters to avoid extreme environmental conditions like drift pack ice, harsh weather conditions, or excessive summer temperatures (Van Guelpen and Davis 1979). In summer months (June to August) in the southern Gulf of St. Lawrence, winter flounder were most abundant in coastal waters (< 40 m deep) and within a temperature range of 3-16 °C (Hanson and Courtenay 1996). As winter flounder abundance began to decline in coastal waters in the fall (September through November), winter flounder became most abundant in the Miramichi Estuary where the temperature was warmer (-1 to 0 °C), and remained in the estuary until spring (Hanson and Courtenay 1996). Contrary to these studies, Ipswich Bay adult winter flounder have substantial inshore-offshore migrations.

In Ipswich Bay, only 20 (5%) winter flounder were recaptured. The majority was caught within the same year they were tagged; most of these fish either remained in the offshore area or exhibited inshore movements identical to the acoustically tracked fish patterns. Unlike previous tagging studies, these fish displayed a wider variance in movements. Several fish were recaptured at the original release location in the following year indicating that winter flounder return to the same spawning areas each year. This has been inferred by others. For example, in the inner New York Bight, 206 recaptures from 7,346 winter flounder tagged at inshore and offshore stations (22 km off coast) showed two inshore areas with separate populations that exhibited a homing pattern during the spawning season (Phelan 1992). However, other fish in this study underwent relatively long distance migrations outside of Ipswich Bay. Three fish were recaptured or relocated via telemetry in Massachusetts Bay (43-57 km away from release site), and two others found on Jeffreys Ledge (22+ km). These movements of these fish warrant further studies into seasonal movements of winter flounder in the GOM to determine home
range size and the degree of mixing of discretely spawning populations, as well as to find out if there is any mixing between stocks.

Limitations with telemetry and flounder

Acoustic tracking has proven very useful for understanding fine-scale movements of fish, and has been used in numerous studies of flatfish, including Solea vulgaris (Lagardere et al., 1988; Lagardere and Sureau, 1989), Platichthys flesus (Wirjoatmodjo and Pitcher, 1984), Paralichthys dentatus (Sackett et al., 2007; Szedmayer and Able, 1993), Pleuronectes platessa (Walker et al., 1978), and Pseudopleuronectes americanus (DeCelles and Cadrin, 2010; Fairchild et al., 2009). However, it is not without limitations, especially when used for tracking flatfish. Unlike round fish which spend proportionately larger (if not all) of their time off bottom, tracking flatfish, which are mostly in contact with the bottom, can be challenging. Signal loss often occurs when an impenetrable object (e.g. a mooring block, large rock, ledge, etc.) blocks the receiver. Such was the case for fish tracked around HSL. Other temporary signal loss occurs due to the burying behavior of the fish (Lagardere and Sureau, 1989). In addition, assumptions derived from telemetry can be strongly based on position of stationary receivers or patterns of manual tracking. For this reason, combining telemetry with other modes of data collection is advisable; in this study trawling was used. Despite these restrictions, telemetry can elucidate fish movement more accurately than other methods. In this study continuous winter flounder movements were tracked for as long as 9 months.

Partnerships:

The collaborative fisherman-scientist partnership was very strong in this project. A total of 5 scientists and 6 fishermen from 3 commercial vessels participated. The project idea actually was conceived through discussions with Carl Bouchard (F/V Stormy Weather). He had been collecting winter flounder in spawning condition each year for several years to be used as broodstock for a UNH winter flounder enhancement project. The flounder always were collected offshore on Jeffreys Ledge. Often they were running ripe. We had a series of conversations about this location and how most people assume winter flounder spawn in estuaries, when his experience had been the opposite. Based on this discrepancy, we chose to pursue the project formally.

The industry partners (Bouchard, Felch, and Goethel) were intricately involved in this project as they helped design the project and methods, create the schedule, and collect the data. In particular, Felch rigged all the hydroacoustic gear required for the project in February 2009. This included picking up receivers from Mass. DMF in Gloucester, purchasing supplies (rope, anchors, chain, blocks, buoys, etc.), constructing highflyers and anchor systems for the receivers, and deploying and moving the gear as needed. All fishermen actively participated each week in the trawl sampling and manual tracking of the fish (March through June 2009). The many discussions about their knowledge of fish assemblages within the study area strongly influenced how this project was carried out. Without their expertise, experience, and boats, this project would not have been possible.
This project has received much interest from other scientists as well. As detailed in the next section, the results may impact winter flounder EFH which would be noticed on many levels.

**Impacts and applications:**
This study contributes to a broader understanding of winter flounder spawning habitat, especially for those populations north of Cape Cod. Given that the long-term viability of marine resources depend on protection of their habitat, and that the Sustainable Fisheries Act requires regional fishery management councils to describe, identify, protect, conserve and enhance essential fish habitats (EFH), studies of winter flounder spawning habitat are necessary. Results of the research are useful to fisheries managers.

Although the role of estuaries in the southern GOM appears to be less critical to spawning winter flounder than originally thought, their importance is not diminished for winter flounder EFH. Just as importantly, adult winter flounder utilize estuaries as post-spawning feeding grounds for several months in summer through fall (Table 4) or until water temperatures exceed their preferred range of 12 to 15 °C (DeCelles and Cadrin, 2010; McCracken, 1963). Post-spawning feeding, or lack thereof, can have dire consequences for female reproductive output in the subsequent year (Burton, 1994; M. J. Wuenschel, 2009). Females that either did not feed to satiation within the six months after spawning or were limited to only 1-2 months feeding time, became non-reproductive (skip spawners) (Burton, 1994). With winter flounder SSB levels at historic lows, it is important to ensure females remain gametogenic each year.

Even if adult winter flounder only occupy estuaries north of Cape Cod after spawning, the estuaries still function as nursery grounds for juveniles. It is well documented that juvenile, and often larval, winter flounder are present in most estuaries regardless of their orientation to Cape Cod (Pereira, 1999). In the southern GOM, juvenile winter flounder reside in estuaries until age 2 before moving out and offshore (Armstrong, 1997; Fairchild et al., 2009; Fairchild et al., 2008).

Conflicts over EFH designations typically revolve around human induced disturbances (dredging, port development) to fish. Based on winter flounder egg EFH designations, activities such as dredging are prohibited during Feb 1 through May 31 in waters ≥ 5 m to protect spawning fish. Based on the results of this study, consideration should be given to extending EFH for GOM winter flounder eggs to deeper waters to protect the predominant coastal spawning populations. Given that spawning season varies by latitude, with northern locations reaching peak spawning later, eliminating the month of February from the no-dredge window likely would have a minimal impact on GOM winter flounder populations.

**Related Projects:**
- Outreach services were provided by NH Sea Grant through Ken LaValley. These services included postcard mailings and poster production.
- This project builds upon and contributes to complimentary adult winter flounder biotelemetry research in and around Plymouth Harbor, MA conducted by Greg DeCelles and Steve Cadrin (UMASS/SMAST). They tagged and released pre-spawning adult fish.
outside of Plymouth Bay in 2007-2009 (DeCelles & Cadrin 2010). The fish were tracked to monitor seasonal movements of adults. Despite historical evidence of spawning inside the harbors, they speculated and showed that the fish may use other locations within the bay for spawning. Both projects sought to clarify misconceptions of the spawning paradigms of the GOM winter flounder stock using identical acoustic equipment. Because of the similarity of the two projects, scientists from both groups met on multiple occasions to share ideas and technology, and DeCelles trained UNH scientists to utilize the acoustic tagging protocol he had developed.

Presentations:


Fairchild, E. A. and W.H. Howell. 2010. Spawning movements of winter flounder in the southern Gulf of Maine. NH Sea Grant Fisheries Round Table Meeting, 26 August 2010, Portsmouth Public Library, Portsmouth, NH.

Fairchild, E. A. 2009. Spawning movements and habitat use of winter flounder in the southern Gulf of Maine. NH Sea Grant Fisheries Round Table Meeting, 24 June 2009, Urban Forestry Center, Portsmouth, NH.


Student Participation:

College Students
Kristin Garabedian (UNH)
Kimberly Little (UNH)
Peter Kirkland (UNH)

Graduate Students
Beth Allison (UNH)
Greg DeCelles (UMASS/SMAST)
Travis Ford (UNH)
Laughlin Siceloff (UNH)
Michelle Walsh (UNH)
Published Reports and Papers:


Informational publications explaining the rationale of the project, who is involved, what to do if a tagged winter flounder is caught, and who to contact for further information. Fishermen were targeted via the following publications:
1. New Hampshire Coastal Conservation Association June 2009 newsletter
2. Mailing to licensed NH fishermen and MA lobstermen
3. Tag-recapture posters at NH and northern MA locations

Images:
Figure 1. The offshore study site located in Ipswich Bay, southwestern GOM, denoted by the red striped polygon. Multiple, overlapping receivers across estuarine inlets created acoustic gates from Kittery, ME south to the Annisquam River, MA. Depth contours range from 18 to 91 m in 9 m increments.

Figure 2. Receiver array configurations: a) original offshore array in place April 5-29; b) reconfigured inshore array in place April 29-May 30. In both panels, the red star and the circles denote the release location and individual receivers, respectively. Depths were 66 m at the release site, 44 to 68 m at the offshore array (a), and 24 to 68 m at the reconfigured array (b).

Figure 3. Adult pre-spawning winter flounder were tagged externally with V9P1L Vemco transmitters based mostly on DeCelles and Cadrin’s (2010) techniques. Pictured here is a male.

Figure 4. The area covered, conservatively estimated as 234 km² based on a detection range of 650 m, by manual tracking with a directional hydrophone over 25 days from April 10 to June 16, 2009. The red star denotes the release location.

Figure 5. Box and whiskers plot showing the size distribution of winter flounder caught in the trawl surveys March – June 2009. CPUE is based on a total of 101 30-min tows in depths ranging from 29-91 meters, with a mean depth of 60 meters.
Figure 6. CPUE and the reproductive status of the female winter flounder population in the study area as determined from the biweekly trawl surveys from March – June 2009. CPUE is based on a total of 101 30-min tows in depths ranging from 29-91 meters, with a mean depth of 60 meters.

Figure 7. Representative inshore movements of 7 acoustically tagged winter flounder from release (red star) to final inshore locations, dates, and depths (end of arrow). HSL = Hampton Shoals Ledge. Additional data are reported in Table 1.

Figure 8. Telemetered tracks of all adult winter flounder that entered estuaries during April – June 2009: a) Fish 682, b) Fish 695, c) Fish 696, d) Fish 708, e) Fish 711, and f) Fish 719.

**Future Research:**
Given the variability of winter flounder movements in the southwestern GOM, further studies are warranted to determine home range size and the degree of mixing of discretely spawning populations, as well as to find out if there is any mixing between stocks. In Ipswich Bay, and other areas of the southern GOM, winter flounder predominantly spawn outside of estuaries. However, exact spawning locations have not been verified, only estimated. Development of some sort of pressure-triggered tag to identify when females spawn, would advance this field substantially. In addition, attention should be focused on Hampton Shoals Ledge, an area identified as a likely spawning ground for Ipswich Bay winter flounder.
Size Distribution (biweekly summaries)

- Early March (n=20)
- Late March (n=5)
- Early April (n=106)
- Late April (n=56)
- Early May (n=59)
- Late May (n=53)
- Early June (n=55)
- Late June (n=17)

Total Length (cm) vs. Time Period

- Values range from 15 to 50 cm
- Data shows variability over time periods